STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

I. Development of the Ovaries and Oögenesis in Moniezia.

C. M. CHILD.

I. Introduction.

Some years ago, during an examination of certain abnormalities [1] in *Moniezia*, the apparent infrequency or total absence of any evidence of mitosis even in regions where rapid growth was taking place attracted my attention. Further investigation showed what various authors had already noted, viz., that anything even remotely resembling mitotic divisions was either entirely absent or extremely rare in most of the growing regions. Nevertheless, a rapid multiplication of nuclei was occurring in these regions as could readily be determined merely by the examination of sections of successive proglottids or more exactly by counting nuclei in well-defined corresponding regions of different proglottids. It was soon possible to establish, beyond a doubt, the fact that the characteristic form of nuclear division was amitotic, not mitotic, no single case of mitosis ever having been seen in most parts of the body.

Naturally the next step was to determine whether the development of the germ cells followed the same course. The present and following papers are devoted to a consideration of the results of these investigations.

The species used are *Moniezia expansa* (Blanchard) and *Moniezia planissima* (Stiles and Hassall) both tapeworms of the sheep, which may be obtained in great abundance from the Chicago Stockyards at certain seasons. The work was begun without any preconceived opinions, but as it progressed it became evident that the facts could not be made to agree with the views commonly accepted among cytologists. For this reason

[1] Child, "Abnormalities in the Cestode Moniezia Expansa, I. and II.," Biol. Bull., Vol. I., Nos. 5 and 6, 1900; III., ibid., Vol. III., Nos. 3 and 4, 1902.

the utmost care as regards observations and conclusions has been taken and publication has been delayed from year to year to permit the examination of new material. In 1904 a brief account of amitosis in the early stages was published.[1] Since that time, however, a large amount of new material has been prepared and much of the old reëxamined, but without essentially altering my conclusions.

Because of the general significance of the data to be described, and in order to forestall possible objections, it has seemed advisable to give in some detail the methods of preparation and procedure. Fresh material was fixed in May or June of four different years. In all, except the first lot of material, the animals were fixed within five minutes after the sheep had been killed. The first material was fixed about two hours after removal from the intestine of the newly killed sheep, but all specimens were alive and apparently in good condition when fixed, and results showed that this material was as satisfactory as that fixed immediately after removal from the host.

A considerable variety of fixing agents was employed in order to discover the best possible fixation and to eliminate the possible effect of particular fixing agents. The fluids used include Hermann's, Chichkoff's, Gilson's, Perenyi's, Merkel's, HNO five per cent., HNO two per cent., HNO two per cent. for one or two minutes followed by Merkel for twenty-four hours or more, aqueous saturated solution of sublimate, sublimate with one per cent. acetic acid and Graf's chrom-oxalic mixture. While certain methods of fixation proved more satisfactory than others the results obtained were essentially identical in all cases so far as the points in question were involved. In the work of the later years Hermann's fluids, chrom-oxalic and aqueous saturated sublimate, were most frequently used as they had been shown to be satisfactory. The chrom-oxalic does not preserve the delicate cytoplasmic structures in the young testes and ovaries as well as does the sublimate, but the nuclear structures are equally clear in both cases.

Various methods of staining were also employed, and while the essential features are visible after almost any fairly good

[1] Child, "Amitosis in Moniezia," *Anat. Anz.*, 1904.

nuclear stain, Heidenhain's iron-hæmatoxylin was most commonly used because of its well-known sharp definition. Undoubtedly this stain is of little or no value for determining differences of chemical composition but it is certainly unsurpassed for bringing out physical differences. Almost the whole body of one worm from the scolex to the region where the uterus contained late cleavage stages was sectioned. From other chains pieces containing three or four proglottids were taken at intervals of from ten to forty proglottids, throughout the body. Pieces separated by thirty or forty or even a larger number of proglottids give stages so near together that nothing is lost, but since it was necessary to make certain that mitosis did not occur periodically in certain regions the intervals between pieces were made much shorter in certain cases. The pieces intervening between those sectioned were numbered and kept and frequently some of them were sectioned later when a larger number of cases of certain stages was needed or some point remained to be settled. Every piece sectioned included at least two complete proglottids and usually more, in the anterior regions often fifteen or twenty. Sections were cut 3–5 μ thick.

New material was sectioned and examined in four different years and each year the old material was reëxamined. Some four hundred camera drawings of nuclei or groups dividing amitotically have been made and in all cases the greatest care has been taken to select those cases which were most clear and convincing. Cases which might possibly be interpreted as amitosis were recorded only when they formed part of a region included in a drawing made for other reasons. The cases recorded by special drawings were usually those which it seemed impossible to interpret in any other way. Many of the cases from which drawings were made were examined by other persons and their interpretation agreed with my own. The observations were all made with a 2 mm. oil immersion lens. With this power some of the divisions may be seen with almost diagrammatic clearness provided fixation and staining are satisfactory.

So far as I am aware I have employed every possible means to establish my observations and to eliminate errors. Amitotic division is not as readily distinguished as mitosis, for there are

no visible characteristic stages of preparation and reconstruction and no clearly visible chromosomes and spindles. The dividing nucleus usually does not stain differently from any other, and after division there is in many cases no demonstrative evidence that the nuclei have arisen by division. Certain critical stages in the division must be found, viz., those in which separation is just beginning and the two parts are manifestly connected. Moreover, such stages must be found frequently in order to establish the presumption that anything more than an abnormal or perhaps a degenerative process is involved. My observations have fulfilled these conditions. Thousands of cases of the critical stages have been observed. Cases more or less similar to every case figured in the drawings have been observed repeatedly. As regards a possible failure to recognize mitoses when they occur it should perhaps be said that perfectly distinct and characteristic mitoses do occur at certain times and places and that there is no chance for confusion of these with anything else: chromosomes, spindle, centrosome, equatorial plate, division of the chromosomes, etc., all are visible and instantly recognizable. In fact it has been possible in the germ cells of one species to establish the number of chromosomes with a considerable degree of accuracy. It is very certain that the form of division which I have designated amitosis in these species cannot be interpreted as mitosis indistinctly visible or of peculiar form. All drawings are made from camera drawings and schematization of the actual cases of division and "improvement" of the camera-drawings have been avoided as far as possible.

Many of the figures are schematic in that non-essentials are omitted and simple methods of representation are employed, but every case of amitosis figured is as nearly like the observed case as it was possible to make it after the most careful examination. No attempt has been made to represent the parenchymal substance in which the cells lie in the earlier stages of development, and when, as is often the case, no well-marked area of cytoplasm appears about the nucleus, no cell-boundaries are indicated and the nucleus alone is drawn. As a matter of fact the distinction between cytoplasm and parenchymal substance, at least during the earlier stages, is not nearly as sharp as the figures indicate, for

no visible cell membrane is present. For the sake of simplicity, however, the approximate area of the cytoplasm about the nucleus is indicated by a line.

To give a detailed description of the development of the reproductive organs in *Moniezia* is beyond the present purpose. The morphological features concern us only secondarily and will be considered only so far as may be necessary for the understanding of other matters.

II. The Nucleus and the Types of Division.

The nuclei of most of the somatic structures and of the young germ cells do not differ widely in appearance. The nucleus contains a deeply staining "nucleolus" which appears to be at least in large part chromatic in composition and might perhaps more properly be called a karyosome. For the present, however, I prefer to use the term nucleolus. After the usual degree of extraction this is very commonly the only element stained in the nucleus which appears entirely homogenous except for this body. With less extraction other granules are visible scattered here and there through the nucleus, but a distinct reticular structure does not appear. This type of nucleus is shown in most of the figures of earlier stages of ovarian development. In various figures there are cases where the nucleus shows a few small granules in addition to the nucleolus (Figs. 8, *A*, *b*, *d*, *e*, etc., 13, *C*, etc.). Nuclei are frequently found with two nucleoli both of which may be of equal size (Figs. 8, *A*, *f*; 14, *A*, *a*, etc.) or they may be unequal (Figs. 14, *D*; 15, *B*, *a*; 16, *b*, etc.). The question as to whether the two nucleoli always arise by the division of one it has been impossible to settle. Sometimes (Fig. 15, *A*, *a*) a minute nucleolus is found apparently in contact with a much larger one and occasionally (Figs. 7, *c*; 9, *B*) two nucleoli apparently connected by a strand of stained substance are seen. On the other hand, in many cases the two nucleoli of very different size are widely separated (Fig. 8, *A*, *a* and *g*) as if one were arising *de novo*. It seems impossible to decide such questions as this until our methods of study of the cell are greatly improved. From my own observations I should conclude provisionally that both methods of origin exist. It is certain, how-

ever, that elongation and division of the nucleolus is not a typical feature of amitosis here. The nucleolus is always spherical or nearly so.

When amitosis occurs each part of the dividing nucleus usually — very probably always — contains a nucleolus. Occasionally it cannot be found but its apparent absence may be due to too great extraction or to loss from the section. It is probable that in *Moniezia* the formation of two nucleoli in a nucleus will be followed sooner or later by division, though division need not necessarily occur at once.

The process of amitosis is simple as far as visible features are concerned, but various apparent modifications occur. In some cases a constriction in the nuclear membrane appears, extending about the whole circumference of the nucleus or limited to one side (Figs. 3, *b*, *c*, *d*, *e*; 5, *a*, *b*; 7, *e*; 13, *A*, *B*., etc.). Frequently there is a faint extension from the deepest part of the constriction partly or wholly across the nucleus (Figs. 3, *b*, *c*; 5, *b*, *c*; 7, *e*, etc.). In other cases the formation of a nuclear plate or membrane across some part of the nucleus takes place before anything more than a very slight constriction appears in the old nuclear membrane (Figs. 7, *f*, *g*; 8, *A*, *e*; 13, *C*; 13, *D*, etc.). In such cases it is possible by careful focusing to follow the new membrane across the whole diameter of the nucleus.

The method of separation of the products of division also varies in accordance with the differences in the earlier stages. The nuclei sometimes separate from one side (Figs. 3, *c*; 5, *c*; 7, *e*; 15, *B*, *b*, etc.), such cases being presumably the result of formation of the constriction from one side. Frequently also the constriction appears to deepen uniformly about the whole circumference (Fig. 3, *d*, *e*, etc.), separation being completed at or near the middle. In those cases where a distinct nuclear plate or partition forms across the whole nucleus separation seems to occur simultaneously or nearly so over the whole surface (Figs. 8, *A*, *c*, *i*; 9, *A*, *b*, etc.). These cases are perhaps the most demonstrative of all, for the flattened surfaces of the two nuclei and occasionally their contact at one margin (Figs. 8, *A*, *c*; 9, *A*, *b*) leave no room for doubt that division has actually

occurred. Occasionally the margins of the two nuclei are the last portions to separate (Fig. 16, *a*). The flattened surfaces are undoubtedly soon lost after division. Some of the cases of separation from one side show very clearly that the separated parts of the surface begin to become convex before separation is completed (Fig. 5, *c*).

The division of the cytoplasm also varies to some extent, but is more difficult to observe since the cytoplasm is usually without any sharply defined boundary. In some cases a constriction of the cytoplasm follows the constriction of the nucleus (Figs. 3, *a*; 7, *f*, *i*; 9, *A*, *d*; 29, *b*, etc.), and in others the nuclear division may be completed before the cytoplasm shows any trace of constriction. (Figs. 7, *d*, *g*; 8, *A*, *c*, *e*, *i*; 11, *A*, *b*; 14, *A*, *b*, etc.). Occasionally nuclear and cytoplasmic division are apparently almost simultaneous (Figs. 7, *h*, *i*; 8, *A*, *h*, etc.). A few cases have been noted where the division of the cytoplasm is "endogenous." Such a case is shown in Fig. 11, *A*, *a*; and another in Fig. 21, *a*. Such cases as Figs. 2, *a*; 5, *d*, and 29, *a*, seem to indicate that a "cell-plate" may sometimes be formed across the cytoplasm, though after the appearance of a cell-plate it is of course impossible to be absolutely certain that the two cells are the product of a division. But the fact that the nuclei with their surrounding areas of cytoplasm are usually isolated renders it probable that such cases represent division.

Not infrequently one part of the nucleus stains more deeply than the other. In such cases the stain is uniformly distributed in each part but the boundary line between the darker and lighter portion is sharp. Figs. 7, *b*, 14, *B*, *a*, and 16, *c*, show cases of this kind. This difference in staining is of some importance as indicating that there are differences of some sort in the two regions and probably also that the two are functioning more or less independently.

Occasionally a case of what appears to be an "endogenous" division is found (Fig. 13, *E*). In such cases the nuclear plate or new membrane does not appear as a simple partition but two distinct membranes more or less convex toward each other are formed, while the old nuclear membrane appears to surround the whole. Some cases of this sort of almost diagrammatic clearness

have been observed and in one of the turbellaria, a form with much larger nuclei, I have recently seen something similar and am therefore inclined to believe that cases of this sort actually do occur. In later stages the old membrane seems to disappear leaving two separate nuclei.

The occurrence of amitosis is probably to be regarded as the result of the establishment of more or less independent functional regions in different parts of the nucleus and the consequent formation of a membrane about each of these. The details of the process must of course differ according to conditions so that many different forms of amitosis may occur, all due primarily to the same factors. The "endogenous" method of division is not perhaps so widely different from the others as might appear. The old nucleus is so large or the new "functional" nuclei so small that parts of the old nucleus are left out when the new membranes are formed. Ordinarily the new membranes are formed in direct contact, here they are merely formed separately.

As regards the process of mitosis but little need be said here. The maturation mitoses will be described later but the tissue mitoses and those in the germ mother-cells differ more or less from these. In preparation for mitosis the nucleus stains more deeply and traces of a spireme are sometimes seen but the preparation first becomes readily recognizable when the chromosomes are formed (Fig. 8, *A*). The largest number of chromosomes counted is fourteen but accuracy is out of the question here. Fig. 8, *B*, shows a case where twelve were clearly seen. A spindle in metaphase is seen in Fig. 8, *A*; it is usually possible to distinguish dark bodies at the poles but much depends on the degree of extraction; the spindle fibers are very delicate and astral radiations are not certainly distinguishable. A later stage is seen in *a*, Fig. 5. Any stage of the division from the formation of the chromosomes to and including the late anaphase is as readily recognizable in the sections as in the figures.

The occurrence of these two forms of division side by side is an indubitable fact, but further data will be given in following papers. There can be little doubt that each form of division is a reaction to special conditions. Certain observations of my own indicate that amitosis occurs more frequently in very rapid and

mitosis more frequently in slower growth. But until other data are described theoretical considerations are out of place.

III. The Early Stages of the Female Reproductive Organs.

The ovary itself does not appear in the earliest stages of development of the female organs. It is in fact formed only after a considerable portion of the ducts has differentiated. The earliest visible stage in the development of the female organs is the increase in the number of nuclei in a region immediately adjoining the longitudinal nephridial canals. Viewed from the surface it appears as in Fig. 1, *A*. A somewhat later stage is shown in Fig. 1, *B*. The nuclei are more closely packed together here

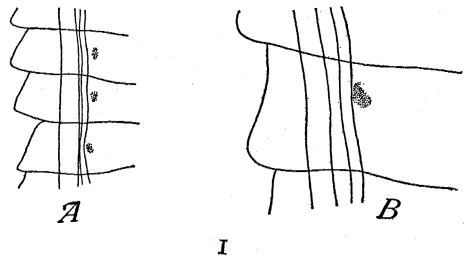


Fig. 1, *A*, 1, *B*.

than elsewhere consequently the region stains more deeply. This region differentiates later on into the middle region of the reproductive ducts. Sections through this region at this stage show a number of nuclei often surrounded by more or less cytoplasm without definite boundary. These nuclei are indistinguishable from the other parenchymal nuclei except that many of them are smaller. Parenchymal fibers can often be traced from the cells and the region is not marked off in any way from the parenchyma.

But the most interesting point forpresent purposes is the apparent absence of mitosis in these regions. Although the writer has examined hundreds of sections of these early stages he has never seen a single case of mitosis. Yet it is very evident that an exceedingly rapid multiplication of nuclei is taking place, for the size of the area and the number of nuclei increases rapidly with

increasing distance from the scolex. Figs. 2 and 3 show small portions of this proliferating region. Fig. 2 is from *M. planissima*, Fig. 3 from *M. expansa*. The cytoplasm about the nucleus

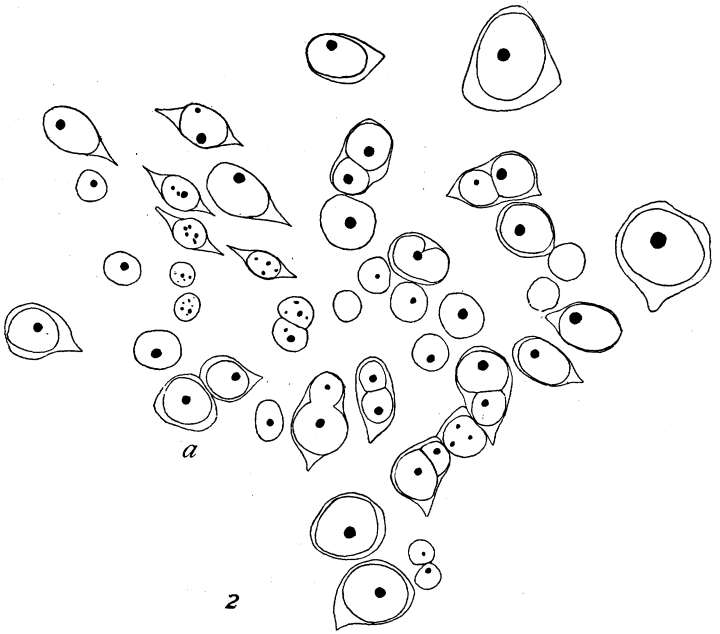


FIG. 2.

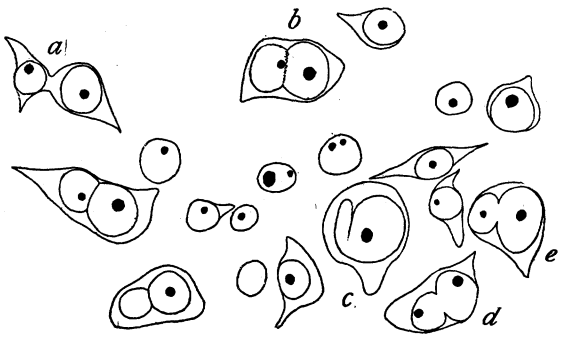


FIG. 3.

is often so slight in amount as to be almost invisible. All the cells lie free in the parenchyma. A number of nuclei in both figures show various stages of amitosis and others have appar-

ently only recently separated. From this stage up to the period when the ducts begin to assume definite form the appearance of these regions is much the same. The course of development of this area certainly favors the view that the proliferation is due to some localized stimulus or condition rather than to anything inherent in the cells themselves. In the central portions of the area the nuclei have divided so rapidly that they are very small; nearer the periphery they are larger and about the outside of the area are nuclei of the same size as other parenchymal muclei. Divisions likewise decrease in frequency from the center toward the periphery. Fig. 2 shows this difference to some extent. The nuclei near the center of the figure which represents approximately the center of the proliferating area are much smaller than those about the periphery. The two large nuclei in the upper right corner are about the size of typical parenchymal nuclei. Fig. 3 is a smaller area from a somewhat later stage and entirely within the proliferating region.

The later development of the ducts will be considered more fully in connection with other somatic structures. It is typically a process of continued amitotic division although in some individuals an occasional case of typical mitosis occurs.

IV. The Formation of the Ovary.

From the region of its first appearance near the nephridial canals (Fig. 1) the proliferating area gradually extends somewhat toward the median plane and toward one surface of the proglottid known as the ventral surface. So far as can be determined there is no appreciable migration of cells through the parenchyma; each part seems to be formed *in situ*, the stimulus to proliferation continually involving more of the parenchymal nuclei and extending in a more or less definite direction.

As the inner and ventral end of the proliferating area approaches the inner layer of circular muscles it spreads out into a flattened somewhat disc-like area exactly as if it had encountered resistance to its growth in the original direction and so had begun to spread out in other directions. Fig. 4 shows a stage soon after the disc-like flattened terminal region has appeared. This disc-like terminal portion indicated by *o* is the ovary. In

connection with it and indicated diagramatically in the figure is the oviduct. The outer portion of the female duct, *i. e.*, that leading from the region of the nephridial canals, is still in an early stage of development and the genital opening has not yet appeared. This sequence in the formation of parts is rather peculiar; the first portion to appear becomes the middle region of the ducts while inner and outer terminal portions, including the ovary appear considerably later.

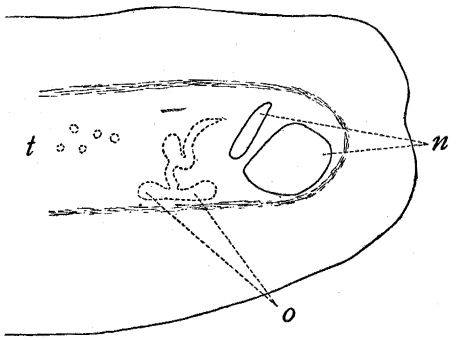


FIG. 4.

In its earliest stages the ovary does not differ very widely in appearance from the early stages of the ducts. It consists merely of a number of nuclei in the parenchyma surrounded by more or less cytoplasm and undergoing frequent amitotic divisions. But the divisions seem to be somewhat less frequent here than in the regions of the ducts and the nuclei never become so reduced in size. But one difference between the ovary and the early stages of duct-formation exists: viz., the occasional occurrence of a case of mitosis in the ovary. Fig. 5 illustrates very clearly the occurrence side by side of the two forms of division. It represents a portion of a longitudinal section through the inner end of the oviduct and the ovarian region. The smaller cells (*od*) on the left in the upper part of the figure with elongated cytoplasmic areas represent the terminal portions of the oviduct and the region between these and the muscles, which are represented by small circles, the ovarian region. In this section only a few of the cells involved in the formation of the ovary appear; they are

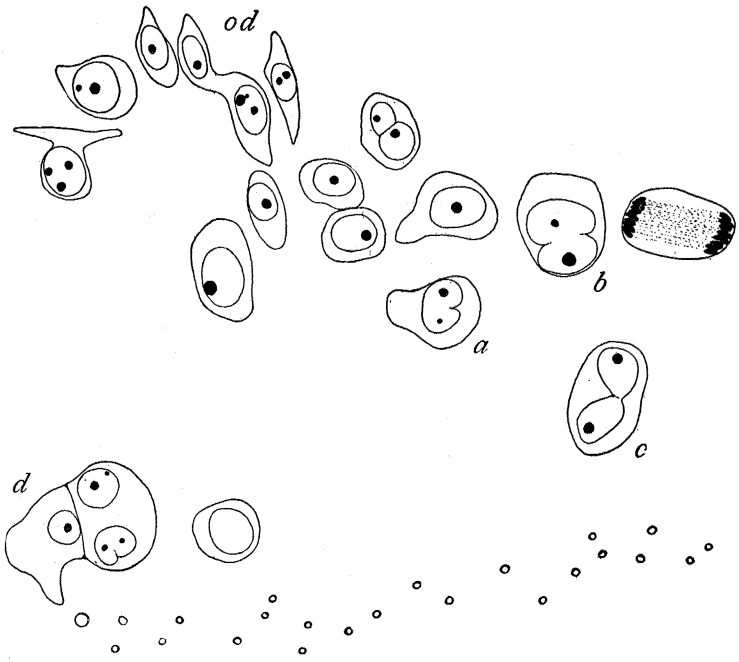


FIG. 5.

mostly in other sections. On the right is a typical case of mitosis in late anaphase ; this is the earliest case of mitosis seen in the ovary ; *c* is a very distinct case of amitosis, *b* is a second case. The smaller nuclei also show two cases of amitosis. Whenever mitosis occurs in the developing ovary other nuclei dividing amitotically are found near it.

V. GROWTH OF THE OVARY.

The further development of the ovary consists in increase in size of the proliferating area and a little later the outgrowth from its margins of finger-like follicles which elongate and give the ovary its characteristic form. A follicular membrane differentiates, apparently from the cytoplasm of the cells about the periphery of the proliferating region, thus separating the ovary from the general parenchyma. Fig. 6 shows a stage in the later development of the ovary in which the follicles have attained almost complete development.

The posterior portion of the disc-like proliferating area which terminates the oviduct at the stage of Fig. 4 is indistinguishable from other parts in the earlier stages but in the later stages shows smaller cells and smaller follicles than the ovary and forms the vitellarium. It is not shown in Fig. 6.

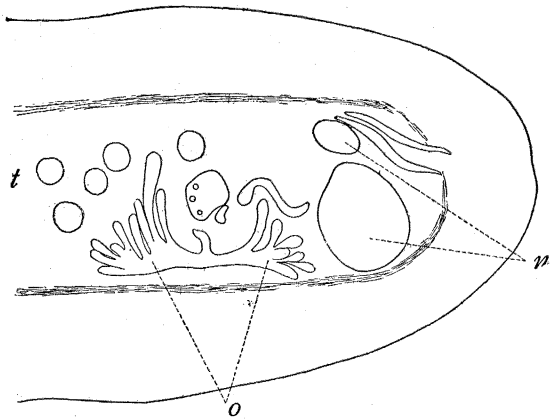


FIG. 6.

After the development of the ovary is completed nuclear division ceases and the mother cells enter upon the first stage of their development as ova. As long as division continues, however, it is predominantly amitotic. The following figures taken from various stages between the formation of the ovary and the completion of its development in both species will show very clearly the prevalence of this process.

During the growth of the ovary the parenchymal substance remaining within the ovary gradually disappears. In many cases, especially after chrom-oxalic, the cytoplasmic areas about the nuclei are indistinct. The cytoplasm is usually not sharply marked off from the parenchymal substance still present so that the whole often appears as a syncytium. After sublimate fixation the cytoplasmic regions appear more distinct. Most of the figures are drawn from sublimate preparations.

Fig. 7 is from a stage slightly later than Fig. 5, before the separate follicles have appeared and before an ovarian membrane has formed. The width of the figure represents the whole

width of the young ovary. In the five nuclei *d*, *e*, *f*, *g*, *i* there can be no doubt regarding the occurrence of amitosis. Each one of these nuclei was examined at all levels and in such cases as *f* and *g* the membrane can be followed through the whole nucleus. The case indicated by *b* is one of those often found where the two parts of the nucleus stain differently. At *f* the two nuclei have apparently recently separated for they are nearly hemispherical in form, their flattened surfaces are parallel and closely approximated and are not visibly covered by cytoplasm.

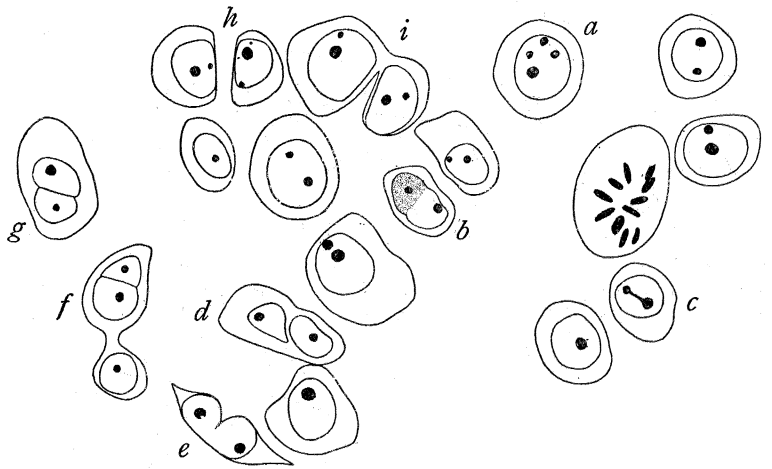


FIG. 7.

The two nuclei at *i* are apparently also the products of a recent division. The cytoplasm is still continuous on the upper side and the nuclei are flattened on opposing faces. The figure also shows one case of mitosis.

Fig. 8, *A*, is from the same proglottid as Fig. 7. At this stage muscle fibres are still visible passing directly through the ovary and are indicated in the figure. It contains six cases of mitosis, the largest number observed in any area of similar size. But in this section are also two very clear cases of amitosis, (*c* and *i*). The cases *b*, *e*, *h* and *j* are also undoubtedly amitoses and several other nuclei in the figure are probably also dividing amitotically. The number of mitoses in this section is of interest since it exceeds so greatly the number seen in any other similar

case. Commonly section after section may be examined without seeing a case of mitosis or occasionally one or two may be found. In the ovary shown in Fig. 8, *A*, however, several consecutive sections showed frequent mitoses particularly in one region of the ovary. Amitosis was common everywhere as

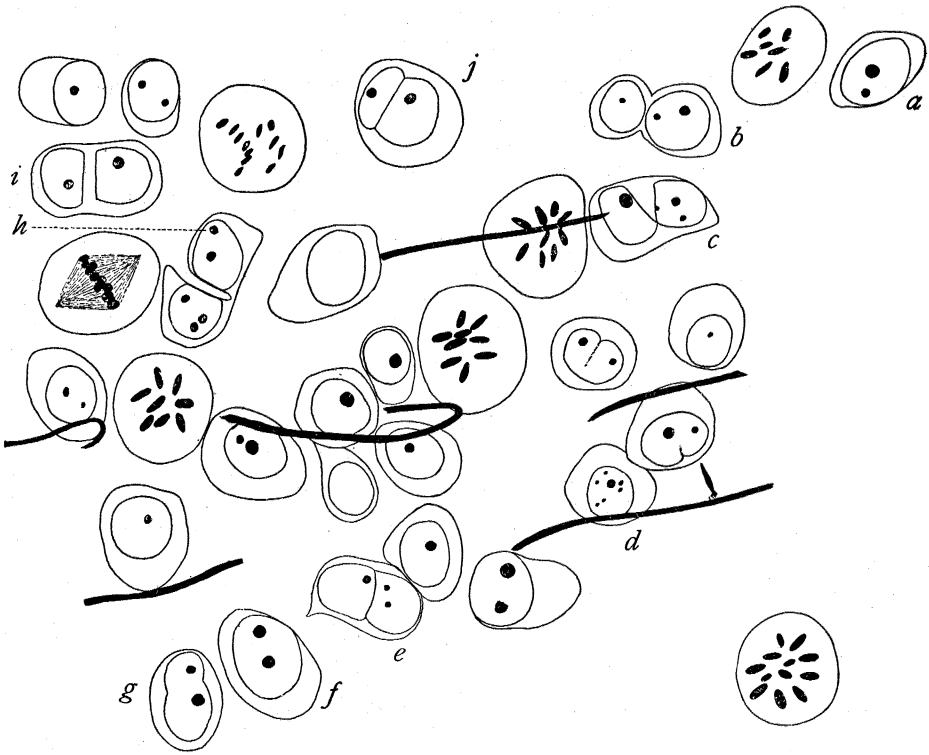


FIG. 8, *A*, 8, *B*.

usual. Discussion as to possible determining conditions is postponed for the present. Fig. 8, *B*, is a case of mitosis in which twelve chromosomes were clearly visible — probably not the whole number. It was usually impossible to determine with accuracy the number of chromosomes in these divisions.

Fig. 9, *A*, is also taken from the same stage and across the whole width of the ovary near its base. At least four perfectly clear cases of amitosis (*a*, *b*, *c*, *d*) are present besides two other

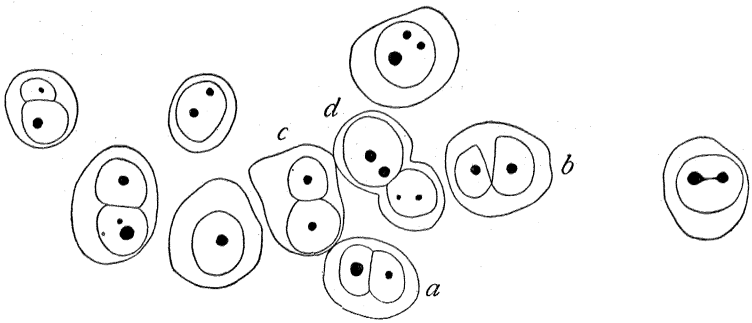
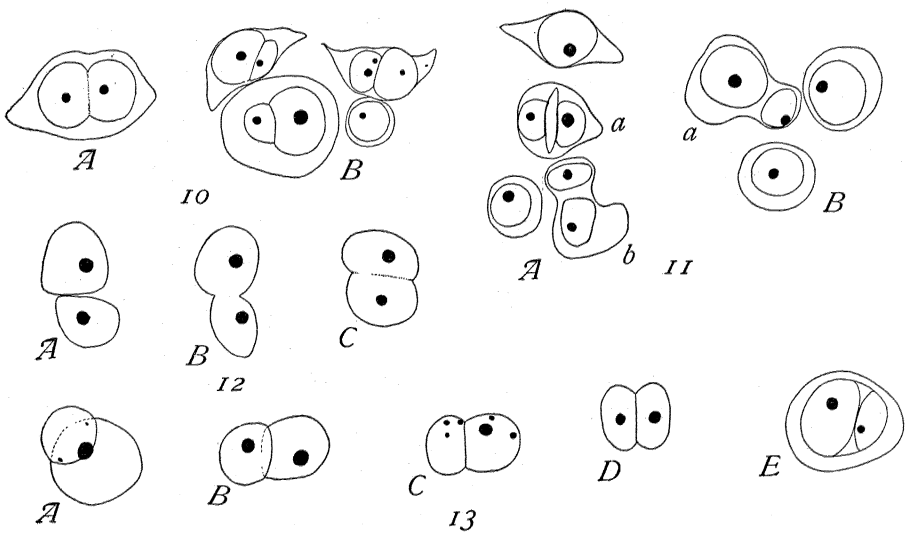


FIG. 9, *A*, 9, *B*.

doubtful cases. Fig. 9, *B*, is a cell from the same ovary with two nucleoli apparently connected.

The Figs. 7–9 are taken from *M. expansa*. Since the course of development is identical in both species so far as can be observed, it is not necessary to duplicate all stages in two species, but a few typical cells and cell-groups from the ovary of *M. planissima* are figured. Fig. 10, *A*, and 10, *B*, both show characteristic cases of amitosis in the earliest stages of ovarian development. Fig. 11, *A*, shows two cases and Fig. 11, *B*, one. The case *a* in Fig. 11, *A*, represents a rather interesting stage



FIGS. 10, *A*, 10, *B*, 11, *A*, 11, *B*. FIG. 12, *A*–12, *C*. FIG. 13, *A*–13, *E*.

and one that possibly does not always occur. The nuclei have separated and between them there is a lenticular space apparently empty, but probably containing fluid in the living condition. This space is apparently still intracellular for the cytoplasm is clearly continuous across its ends. In most cases of amitosis the two nuclei separate at one end first so that no such space is formed.

In Fig. 12, *A* and *B*, the same nucleus is shown at different levels, *A* being the upper portion and *B* the lower. *C* is probably an early stage of amitosis. Both are from slightly later stages of development than Fig. 11.

Cases from a still later stage are shown in Fig. 13, *A–E*. The chain from which Figs. 10–13 were taken showed fewer mitoses in development of all organs than any other examined. Mitosis was scarcely ever seen in the ovary. That individual differences do exist in this respect can scarcely be doubted.

After the individual follicles begin to form, the divisions seem to be more frequent in them, and especially near their tips, than in the central portions of the ovary. Fig. 14, *A–D*, show groups with characteristic amitoses from the developing follicles of *M. expansa*. Fig. 15, *A*, and 15, *B*, are similar, the latter showing a few nuclei from the extreme tip of the young follicle not yet enclosed by a follicular membrane. In Fig. 16 the terminal portion of another follicle is shown with three cases of amitosis.

An early stage in the development of the follicles in *M. planissima* is shown in Fig. 17. The numerous cases of amitosis are clearly visible. At *a* in the left follicle is what appeared to be a group of small nuclei. It may be a multiple amitosis or may possibly represent a stage in reconstitution after mitotic division. The large nuclei in two of the follicles are frequently found along the axes of developing follicles. One of them (*b*) is apparently dividing into three parts. The nuclear divisions which have been described thus far are in reality the oögonial divisions. The amitotic divisions certainly constitute a normal feature in the history of the ova, as there is no evidence that the nuclei which have divided mitotically have a different fate from the others. The relative frequency of mitoses varies not only in different chains

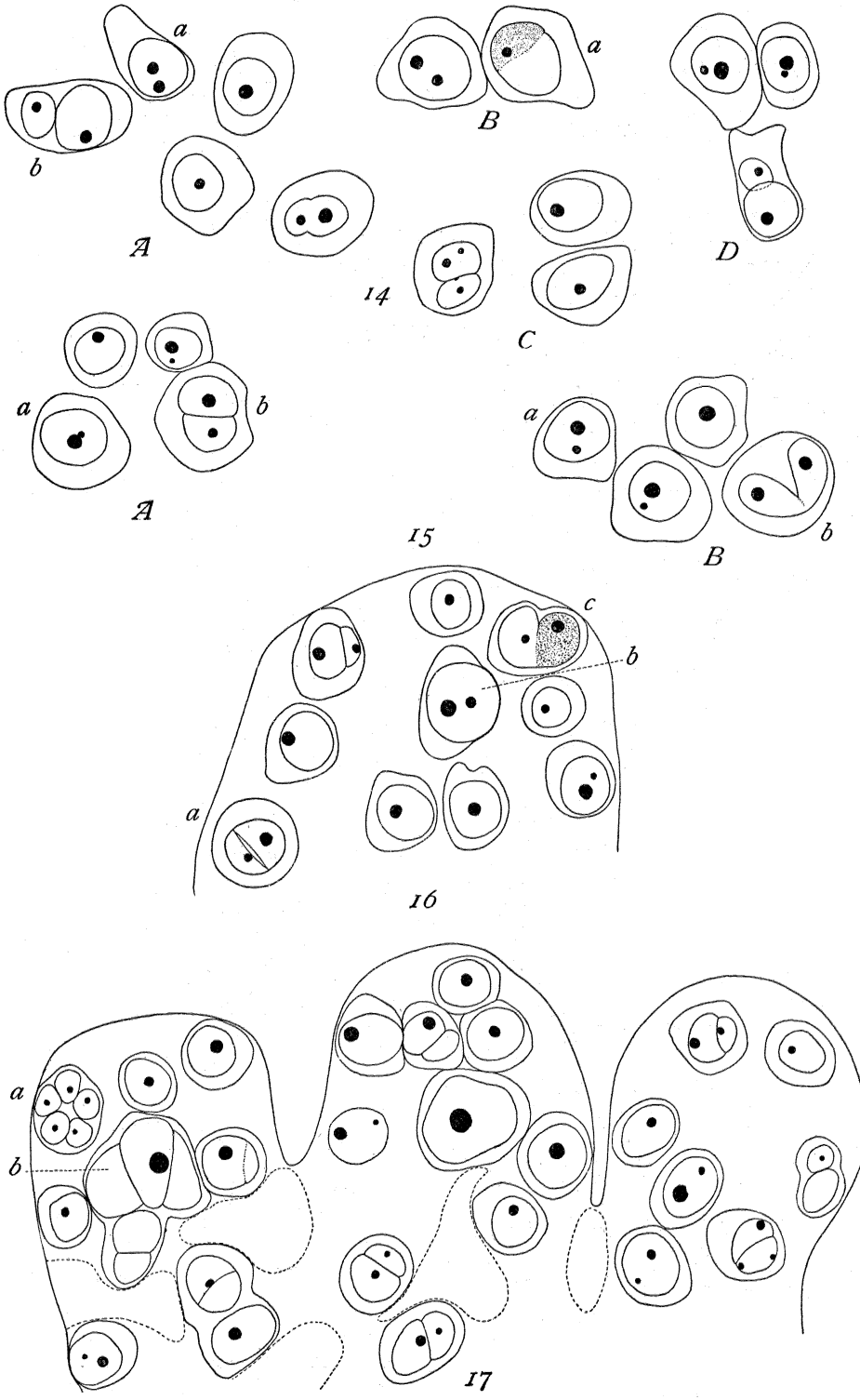


FIG. 14, *A*–14, *B*. FIG. 15, *A*, 15, *B*. FIG. 16. FIG. 17.

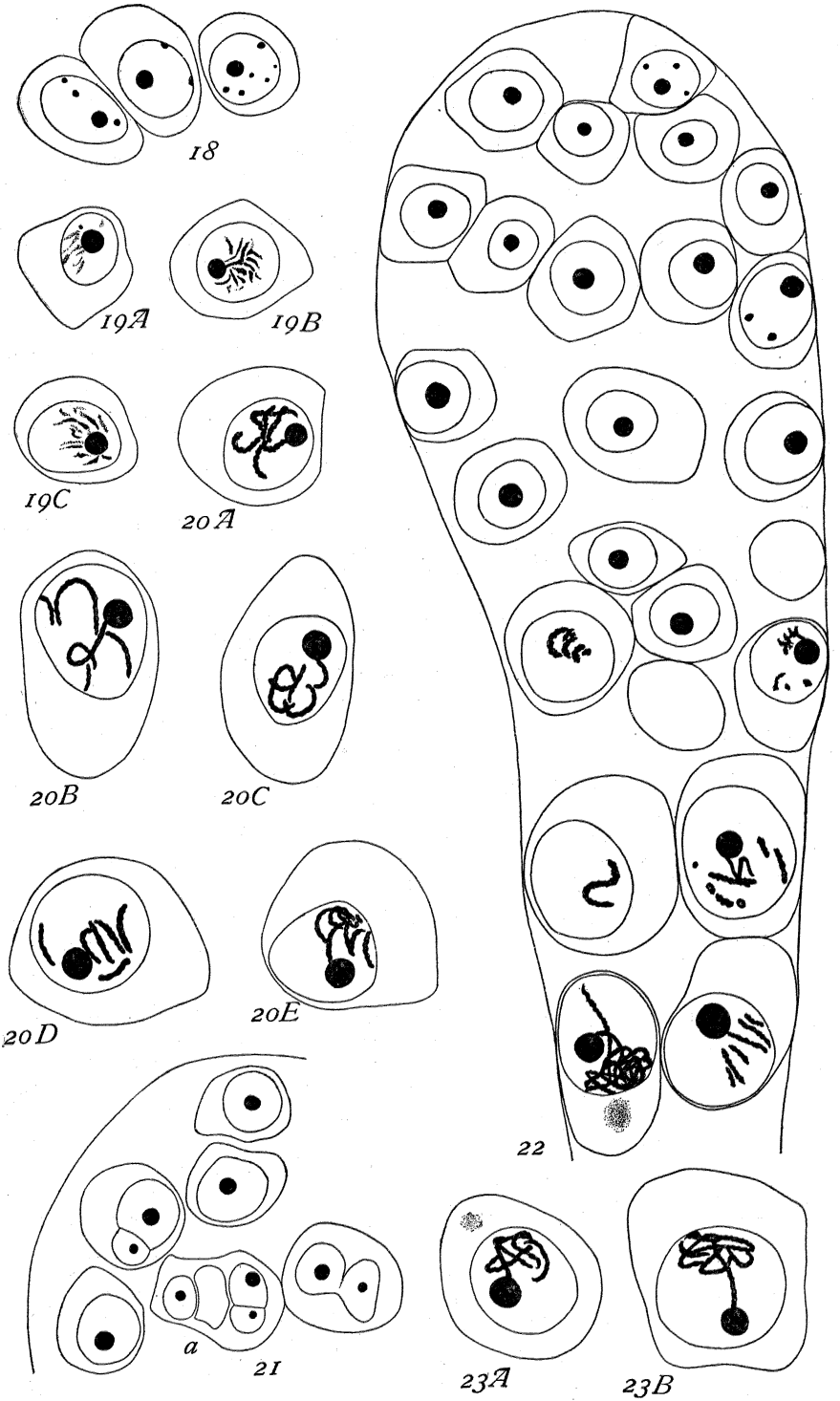


FIG. 18. FIG. 19, *A*, 19, *B*. FIGS. 19, *C*, 20, *A*. FIG. 20, *B*, 20, *C*.
FIG. 20, *D*, 20, *E*. FIG. 21. FIG. 22. FIG. 23, *A*, 23, *B*.

but in different proglottids. The chain in which mitosis was almost never seen in the ovarian development produced apparently as many eggs as others and these developed in the normal manner in the uterus.

VI. The Growth Period, "Synapsis" and Yolk Formation.

Although the later stages in the formation of the ova are not concerned directly with amitosis a brief description of these stages is added in order to demonstrate that nuclei which have passed through a long series of amitoses are quite capable of exhibiting the characteristic phenomena of typical germ cells. These stages in the development of the ova also appear to be identical in both species. All the figures except 22, 23 and 30, *A–D*, are from *M. expansa*.

The oögonia at the end of the period of division are seen in Fig. 18. There is little difference in appearance between them and the dividing cells during ovarian development. The amount of cytoplasm is perhaps slightly greater, but this difference is not marked.

But now the nuclei undergo a sudden and remarkable change. The only deeply staining portions of the nucleus up to this time have been the nucleolus and frequently a few other granules (Fig. 18). Now the nucleus develops rapidly a large amount of chromatin. The earliest observed stages in this development are shown in Fig. 19, *A*–19, *C*. This change in the nuclear substance is accompanied by a great increase in size of both nucleus and cytoplasm. The chromatin soon shows itself in the form of a typical spireme (Figs. 20, *A*–20, *E*, 23, *A*, 23, *B*) which is commonly massed at one side of the nucleus (Figs. 20 *A*, 20, *C*, 20, *E*, 23, *A*, 23, *B*) and in most cases is visibly connected with the nucleolus. So far as can be determined the spireme does not appear to be formed from the substance of the nucleolus, since the latter increases in size like the other elements of the cell. This is clearly a typical case of what is commonly known as synapsis.

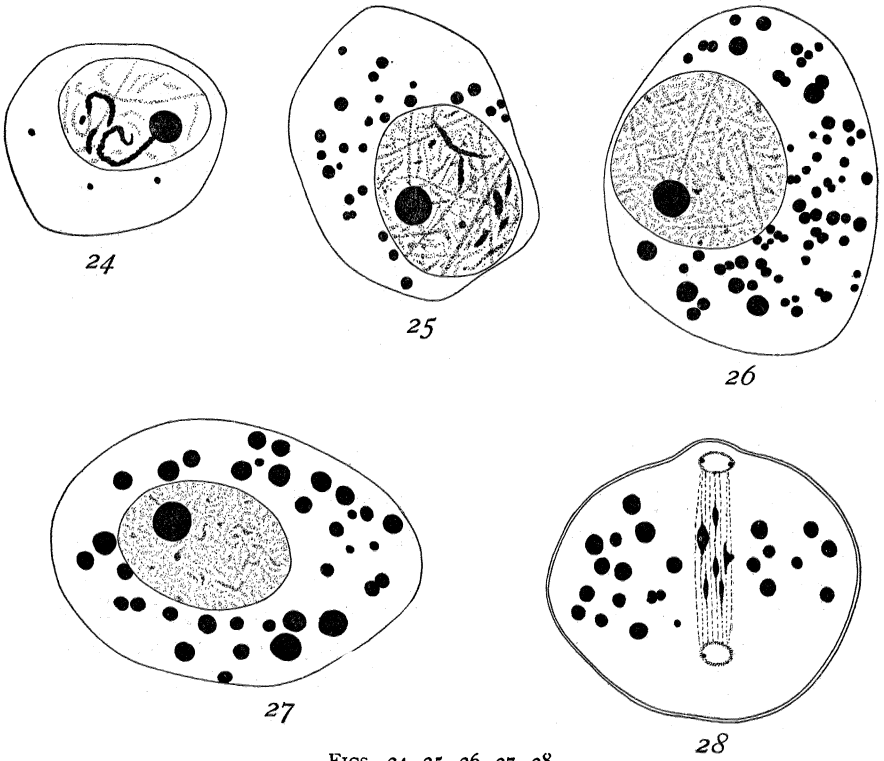
The appearance of these stages varies considerably with the degree of extraction of the stain. If extraction is carried farther than in Fig. 20 only small portions of the spireme or often only

the karyosome retain the stain and the presence of the spireme would not be suspected. The writer is firmly convinced by long experience with this stain that it is of little or no value in determining differences of chemical constitution; size, density, and permeability seem to be the chief factors determining which parts shall retain the stain.

The spireme appears first in the central portions of the ovary adjoining the end of the oviduct and proceeds in all directions distally along the follicles. The follicle tips continue, however, to divide amitotically for some time after the other portions of the ovary have entered the spireme-stage. Fig. 21 shows the tip of a follicle at this stage. In the same ovary the cells near the oviduct were like those in Fig. 20. Fig 22 is a nearly longitudinal section through the terminal portion of a follicle showing the different stages at different levels. In all but one of the five cases with spiremes most of the chromatin is outside the plane of section. In this ovary all the cells have entered the spireme-stage except those in the terminal region of the follicles and division has already ceased there.

Together with the nuclear changes occur marked changes in the cytoplasm. The amount of cytoplasm increases in marked degree as the figures show. It also stains more deeply than before. During synapsis the nucleus is usually more or less asymmetrical in position. In many cases at least the greater amount of cytoplasm is on that side of the nucleus against which the spireme is massed. (Figs. 20, *A*, 20, *C*, 20, *D*, 20, *E*, 23, *A*, 23, *B*). It cannot be stated positively that this is always the case but it is certainly of frequent occurrence. After fixation in chrom-oxalic a regional differentiation of the cytoplasm is visible in many cases and is perhaps a characteristic feature. As noted above, the spireme usually becomes massed at one side of the nucleus: this is followed by the appearance in that part of the cytoplasm nearest the spireme of an area staining more deeply than any other part of the cytoplasm (Fig. 24, *A*). If extraction is carried too far this area is scarcely or not at all visible. It is comparable to certain of the differentiations which have been called yolk nuclei in other eggs and its appearance is followed almost immediately by the formation of yolk granules which in *Moniezia*

are contained in the egg-cell itself. The first yolk granules to appear, however, are not confined to this region but are more or less scattered (Fig. 24). The natural conclusion from the sequence of events is that the change in nuclear condition is in some way correlated with the cytoplasmic changes and since the former precedes, that it is in some way responsible for the latter. Nuclear changes connected with yolk-formation have been de-



FIGS. 24, 25, 26, 27, 28.

scribed by many authors but it is necessary to review the various accounts. It seems probable these nuclear changes indicate an alteration in the metabolic processes and that they are concerned primarily with the increase of the cytoplasm and the deposition of yolk.

Figs. 24–27 show successive stages in yolk-formation. The granules formed first increase in size and others appear. Fusion

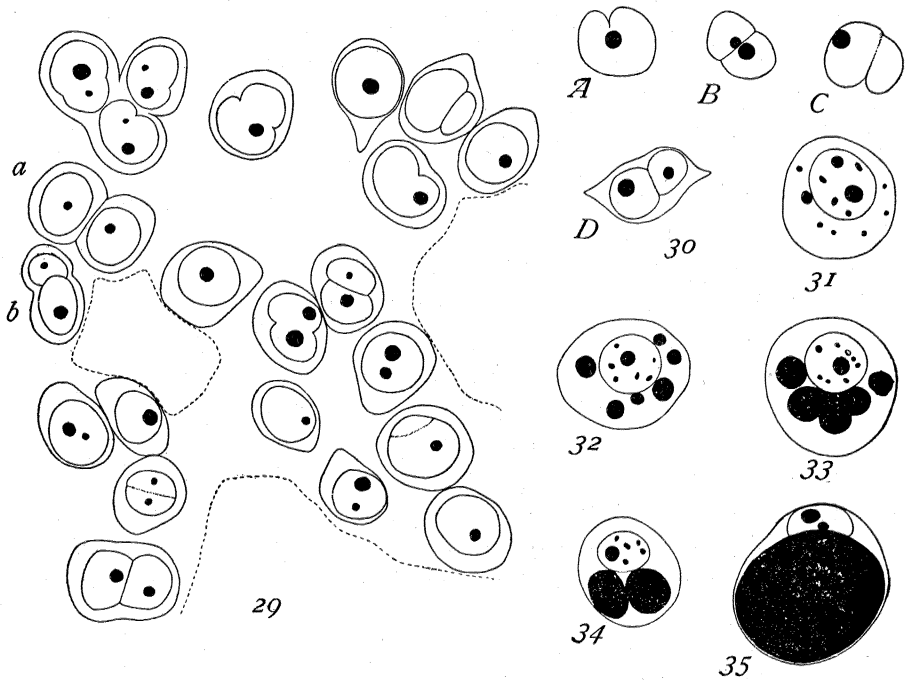
of the smaller to form larger masses also occurs frequently. During yolk-formation the nucleolus remains apparently unchanged, but the spireme soon loses its deeply staining character and its substance appears to spread throughout the nucleus in a more or less recticular condition so that the nucleus resembles the nuclei of most eggs before maturation.

It is almost impossible to reproduce the nuclear structure of this period with any degree of exactness: moreover, it is not certain how far the visible structure is characteristic of the living cell and how far it is the product of fixation. An attempt has been made, however, in Figs. 24–27 to indicate the changes in nuclear structure. Although the spireme of earlier stages has disappeared the nucleus differs in appearance from the ovarian nuclei before the growth period in that a reticulum is now visible while in the earlier stages the nuclei were almost entirely homogenous in appearance except for the nucleolus and other granules. An account of maturation and fertilization will be given in another paper. At this time it need only be said that typical maturation spindles appear, but that it has been impossible to follow the process of chromosome-reduction. Fig. 28 shows the first polar spindle with its enormous centrosomes.

VII. The Development of the Vitellarium.

The "yolk-gland" differentiates from the posterior portion of the ovarian mass of proliferating cells. During the earlier stages its cells are indistinguishable from those of the ovary, but later they can be distinguished by their slightly smaller size. The nuclear division is almost wholly amitotic. Occasionally, however, though less frequently than in the ovary, a case of mitosis is seen. Division seems to be somewhat more rapid than in the ovary, amitoses being commonly more numerous in a given area. As in the ovary the nuclei of earlier stages are surrounded by very little cytoplasm and lie in the parenchymal tissue. Fig. 29 represents a group of nuclei at the stage when the yolk-gland is first distinguishable from the ovary. Numerous amitoses are visible. Fig. 30 shows several cases of division from a later stage. The process of yolk formation in these cells differs from that in the egg cells. It is not preceded by any marked increase in size of the

nucleus or cytoplasm. No spireme has been observed but a number of deeply staining granules appear in the nucleus in addition to the karyosome. The yolk appears first in the form of small granules which increase in size and fuse, until the cell contains a single large spherical mass of yolk and the greatly reduced nucleus is flattened at one side. Successive stages of yolk-development are shown in Figs. 31–35. These cells apparently



FIGS. 29–35.

arise from the same primordium as the ova but have become specialized in the direction of yolk-production. The process of yolk-formation is not identical with that in the egg but this difference is probably correlated with the extreme specialization.

VIII. CONCLUSION.

Extended discussion is better postponed until other data have been presented, but the most important facts of this paper may be briefly stated as follows.

Nuclear division in the development of the female reproductive organs of *Moniezia expansa* and *M. planissima* is predominantly amitotic though typical mitoses occur, their frequency varying apparently in different chains and in different proglottids.

After the long period of repeated amitotic division the nuclei pass through the characteristic mitotic maturation divisions and the cells form typical ova.

The process of amitosis consists in the formation of a constriction in some part of the nucleus or of a "nuclear plate" or membrane across some portion of the nucleus and the separation of the nuclei thus marked off and later of the cytoplasm about each. Each part thus separated usually possesses a visible nucleolus at the time of formation of the membrane.

The fact that the female germ-cells may arise by a long series of divisions almost wholly amitotic is of considerable theoretical importance. There is no room for doubt that the fate of a cell may be the same, whether it divides mitotically or amitotically during developmental stages. It is also very difficult to understand how anything like individuality of the chromosomes can be maintained in this case during the development of the germ-cell.

The two forms of division may occur side by side in the same tissue and at the same stage, but their relative frequency may vary in different individuals and in different proglottids. It seems probable, therefore, and other data will confirm this conclusion, that the form of division is determined by the conditions to which the cell is subjected.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
August, 1906.